The *in vitro* Antioxidant Activity of *n*-Hexane Extract of *Jatropha curcas* L. Leaf

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***Abstract*— Oxidative stress is one of the well-recognized cause of many degenerative diseases. The antioxidant activities of many medicinal plants have been evaluated to obtain the alternative medicines for the prevention of oxidative damages. *Jatropha curcas* L. is a widely used plant. It has been used to treat many diseases. However, the antioxidant activity of its *n*-hexane extract has not been reported. Therefore, this study aimed to evaluate the *in-vitro* antioxidant activity of *n*-hexane extract of *J. curcas* L. leaf using DPPH free radical scavenging activity assay, reducing power activity assay and lipid peroxidation inhibition assay. The antioxidant activity of the extract was compared with trolox as reference standard. *n*-hexane extract of *J. curcas* L. leaf was found to be more capable in inhibiting lipid peroxidation process (IC_{50}=56.629 µg/ml±2.145) than to scavenge DPPH· free radical (IC_{50}=300.834 µg/ml±4.571). Data from reducing power activity assay indicated that this extract possessed higher reductive potential in inducing the $Fe^{3+} \rightarrow Fe^{2+}$ transformation compared with control (P<0.05). In conclusion, the *n*-hexane extract of *J. curcas* L. leaf possessed antioxidant activity.**

Keywords—Jatropha curcas; antioxidant activity; n-hexane extract.

I. Introduction

Cells in our body use oxygen to generate energy and free radicals are produced as a consequence of this process. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are 2 products generated by the cellular redox process. They can be toxic in our body at high concentration, resulting in oxidative stress which might trigger many degenerative diseases such as cancer, arthritis, autoimmune disorders and cardiovascular ([1]). The continuous formation of free radicals in our body is normally controlled by endogenous antioxidants through inhibition or delay the oxidation of lipids or other molecules. This action inhibits the initiation or propagation of oxidative chain reaction through one or more of these mechanisms including reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen ([2], [3]).

Jatropha curcas L. or Thai local name, "saboodum", is a multipurpose drought resistant plant in the Euphorbiaceae family. Having high economic utility, it is widely grown in Thailand and other countries. The seed of *J. curcas* contains high quality oil which can be primarily used as biodiesel. Other parts of this plant especially leaf, however, have been proven to be capable in treatment of many diseases. The leaf of *J. curcas* possesses many chemical compounds. Flavonoid, apigenin, vitexin, isovitexin, sterols and triterpenes have been identified ([4]). The specific compound, a complex of 5-hydroxypyrrolidin-2-one and pyrimidine-2,4-dione has also been isolated from the leaf. This compound has not been discovered in other Euphorbiaceae plants ([5]). Flavonoids, saponin and plant sterols are also effective compounds to produce antioxidant activity ([6]-[8]). However, the antioxidant activity of *n*-hexane extract of *J. curcas* L. leaf has not been examined. Therefore, this study was conducted to assess the antioxidant capacity of *n*-hexane extract of *J. curcas* L. leaf as compare to the antioxidant reference standard.

II. Materials and Methods

A. Reference Standard and Chemicals

Trolox, ascorbic acid, 2,2-diphenyl-1-picrilhydrazyl (DPPH), bovine brain phospholipids extract type VII, thiobarbituric acid (TBA), 2,6-di-tert-butyl-p-cresol were purchased from Sigma (Sigma-Aldrich, GmbH). Polyoxyethylenesorbitan monolaurate (tween 20) were obtained from S.Tong Chemical Co.Ltd, Thailand. All other chemicals used were analytical grade and purchased from either Merck or Carlo Erba.

B. Plant Material

J. curcas L. fresh leaves were collected from Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom Province, Thailand. The plant was taxonomically authenticated by Assoc. Prof. Rungravi Temsiririrkkul. A representative voucher specimen was deposited at Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University.

C. Preparation of Plant Extract

The *n*-hexane extract of *J. curcas* leaf was kindly provided by Assoc. Prof. Rungravi Temsiririrkkul. Fresh leaves of *J. curcas* L. were washed, dried in a hot air oven and pulverized. The dried powder of leaves (1.5 kg) was percolated with *n*-hexane solvent. The hexane was then removed from the filtrate using a rotary vacuum evaporator. The concentrated extract was finally evaporated in the fume hood to obtain the crude extract (37.97 g).

D. Free Radical Scavenging Activity Assay

The DPPH free radical scavenging method was used to evaluate the antioxidant capacity as described by Brand-Williams et al ([9]) with slight modification. The 0.2 mM DPPH solution was prepared by weighing 7.9 mg of DPPH and dissolving it with methanol up to volume 100 ml quantitatively. Stock solutions (3 mg/ml) were prepared by dissolving 75 mg of crude extract in 25 ml of 10% v/v tween 20 in methanol. The stock solution was filtered with 0.45µm filter syringe before diluting with methanol to obtain the working solutions of different concentrations (300-1800 µg/ml). One ml of each working solutions was mixed with freshly prepared 0.2 mM DPPH solution (2 ml) to obtain the final concentrations of 100-600 µg/ml, then incubated in the dark room at room temperature for 30 minutes. After incubation, the absorbance of each solution was measured at 516 nm using UV-VIS spectrophotometer (Shimadzu L-2600). Different concentrations (1-10 µg/ml) of trolox was used as reference standard. Corresponding blank was prepared by adding vehicle to 2 ml of 0.2 mM DPPH solution. Triplicate samples were used in this assay.

The DPPH scavenging capacity of each sample was expressed as percentage of inhibition calculated by the following equation :

DPPH scavenging capacity (%) = (Ab-As)/Ab x 100

Where, Ab = absorbance of the blank
As = absorbance of sample

The percentage of DPPH scavenging activity versus concentration of sample was plotted and calculated to obtain the IC_{50} value (concentration of sample which exerted a 50% of DPPH scavenging activity) using Graphpad Prism version 5.0. The percentage of DPPH scavenging activity was expressed as mean IC_{50} value ± SEM.

E. Reducing Power Activity Assay

A slight modification of the method described by Oktay *et al* and Chayarop *et al* ([10, [11]) were applied to determine the reducing power activity of *n*-hexane extract of *J.curcas* L. leaf. A stock solution of extract was prepared by weighing 140 mg of crude extract and dissolving with 10% v/v tween 20 in methanol up to volume of 10 ml quantitatively. The stock solution was then filtered using a filter with pore diameter of 0.45 µm. 0.5 ml of each working solution was mixed with 0.5 ml of 0.2 M sodium phosphate buffer (pH 6.6,) and 0.5 ml of 1% potassium ferricyanide. After shaken well, the mixture was incubated at 50°C for 20 minutes. The reaction was stopped by adding 2.5 ml of 10% TCA. Then, it was centrifuged at 650 rpm for 10 minutes. 0.5 ml of the supernatant obtained was mixed with 0.4 ml of deionized water and 0.1 ml of 1% $FeCl_3$. The final concentration obtained was 50-1000 µg/ml. Trolox (50-1000 µg/ml) was used as a reference standard. Corresponding blank was prepared using vehicle in the absence of extract or standard. The absorbance was measured spectrophotometrically at 700 nm. Increased absorbance represents an increase in reducing power. Triplicate samples were used for each assay. The results were expressed as mean values ± SEM. The absorbance of each sample at 700 nm was plotted against each concentration of samples.

F. Non-enzymatic Lipid Peroxidation Inhibition Assay

The extent of inhibition on lipid peroxidation was determined using TBARS assay as described by Burits and Bucar ([12]) and Siriwatametanon et al ([13]) . Liposomes were prepared from bovine brain

extract type VII by mixing with 5 mg/ml PBS pH 7.4 and homogenizing under cold temperature until obtaining a milky solution. The reaction solution in a total volume of 100 µl contained 50 µl bovine brain extract suspension, 30 µl extract compound dissolved in PBS/tween 20, 10 µl 1 mM $FeCl_3$ and 10 µl 1 mM ascorbic acid. All reagents were freshly prepared. This solution was shaken well. After incubation at 37°C for 1 hour, TBA (1% in 50 mM NaOH, 100 µl), TCA (2.8%, 100 µl) and 2,6-di-tert-butyl-p-cresol (2%, 10 µl) were added into the sample solution and incubated at 80°C for 20 min. After cooling down for 10 min, 250 µl of 99% *n*-butanol was added and shaken well. The solution was then centrifuged at 3500 rpm for 5 min. The absorbance of supernatant (100 µl) was measured at 534 nm. A blank was prepared without adding Fe-ascorbate while a control was obtained by only adding–vehicle. Trolox was used as a reference standard. The percentage of inhibition (I) of lipid peroxidation was calculated according to the following equation:

$$I\ (\%)=(Ac-As)/Ac \times 100$$

Where Ac was the absorbance of the control while As was the absorbance of the sample containing the inhibitor.

G. Statistical Analysis

The data were analyzed statistically using Student's unpaired t-test to determine the different between two groups whereas multiple comparisons were analyzed by one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test. Data were analyzed by SPSS (version 17.0 for windows). The IC_{50} values were calculated using Graphpad Prism version.5 software for windows. All data are expressed as mean ± SEM.

III. Results and Discussion

A. Free Radical Scavenging Activity Assay

In this study, the stable nitrogen free radical, DPPH•, was used to test the radical scavenging activity of *n*-hexane extract of *J. curcas* L. leaf. This method is widely used and considered as one of the standard and easy colorimetric assays to evaluate the antioxidant activity of pure compounds ([14]). Another advantage of DPPH• scavenging activity assay is that it is unaffected by certain side reactions, such as metal-ion chelation and enzyme inhibition. The principle of this method was based on the antioxidant molecules of compounds in the plant which can donate a hydrogen (H) atom to DPPH• radical of DPPH solution, resulting a non-radical form of $DPPH_2$ molecule. This reaction faded purple colour of DPPH solution or changed it to yellow in the presence of antioxidant ([10]). The amount of reduced DPPH• was monitored spectrophotometrically at 516 nm. Figure 1 illustrated the scavenging activity of trolox and extract. Both trolox and extract reduced DPPH• stable free radical in a dose dependent manner. The values of IC_{50} of trolox and extract were 5.0937 µg/ml ± 0.046 and 300.834 µg/ml ± 4.571 respectively. The extract exhibited less potent scavenging activity than trolox. It might be explained that the amount of hydrogen donor molecules in this non-polar extraction of *J. curcas* L.leaf was low.

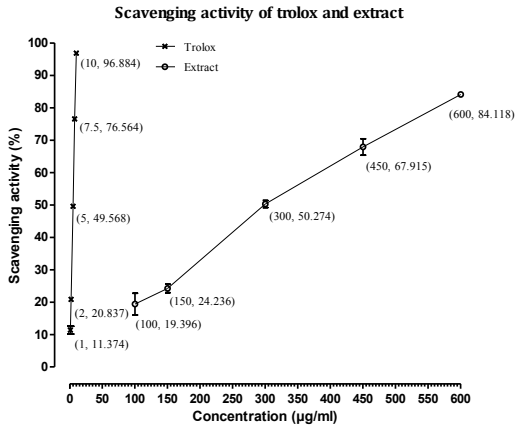

Fig. 1. Free radical scavenging activity of trolox as reference standard (n=3) and extract (n=3) at different concentrations. Each value is expressed as mean ± SEM.

B. Reducing Power Activity Assay

The activity of ferric ion (Fe^{3+}) reduction was determined according to this assay. Different from DPPH• radical scavenging activity, this assay was carried out to assess electron-donating activity of an antioxidant compound as a reductant. This activity leads the reduction of the Fe^{3+} from ferrycyanide complex to the ferrous (Fe^{2+}) form. Moreover, the

yellow colour of ferricyanide complex changed to various colour of green and blue depending upon the reductive ability of each antioxidant compound in the sample. Therefore, the ferrous form was measured spectrophotometrically by the formation of Perl's Prussian blue at 700 nm ([10], [15], [16])

The reducing power activity of *n*-hexane extract of *J. curcas* L. leaf and trolox are illustrated in Fig.2. All concentrations of either extract or trolox revealed higher reducing power activities than control and these differences were statistically significant (P<0.05). It indicated that both samples had an antioxidant activity which may be related to its electron donating ability. The extract as well as trolox showed a dose-dependent reducing power at concentrations ranging from 50-1000 µg/ml. Nevertheless, the antioxidant activity of extract was lower than trolox and its activity was significantly difference (P<0.05). This less potency may be attributed to a small amount of phenolic compound in the extract ([17]) since phenolic antioxidant activity has an important role in electron-donating activity of this assay ([18]).

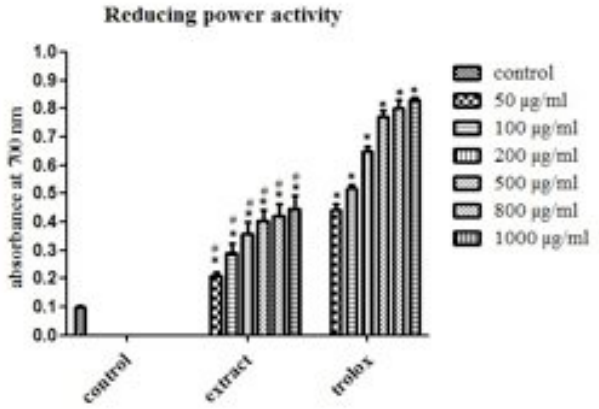

Fig. 2. Total reductive potential of different concentrations of trolox and n-hexane extract of J.curcas leaf. Compared with the control using spectrophotometric detection of the Fe3+ - Fe2+ transformations. **P* < 0.05 compared with the control. #*P* < 0.05 compared with the same concentration of trolox. Each value is expressed as mean ± SEM (n=3)

C. Non-enzymatic Lipid Peroxidation Inhibition Assay

This assay was conducted to assess hydroxyl radical (OH•) scavenging activity of the antioxidant compounds in a sample. The OH• was induced by adding ferric ion of $FeCl_3$ in the presence of ascorbic acid as a reducing agent. Bovine brain extract was used as liposomes in a lipid peroxidation model system ([19]). This reaction produced malondialdehyde (MDA) and other aldehydes that reacted with TBA after heating for 20 min and resulted a solution with pink colour ([20]). The amount of MDA and other aldehydes produced was measured spectrophotometrically at 534 nm.

Both trolox and the plant extract inhibited Ferric ion-ascorbate-induced peroxidation of liposomes in a dose-dependent activity but trolox was more potent than extract as shown in Fig. 3. The IC_{50} values of trolox and extract were 5.935 µg/ml ± 1.176 and 56.629 µg/ml ± 2.145 respectively. Therefore it was shown that the antioxidant activity of extract was higher in lipid peroxidation assay than DPPH• radical scavenging assay.It might be related to the non-polar compounds, phytosterols, extracted in percolation process such as β-sitosterol. Regarding to the previous report, the *n*-hexane extract of *J. curcas* leaf contained β-sitosterol as a main compound ([17]). Therefore, this extract should possess high capability to scavenge OH• radical in lipid peroxidation process ([19]).

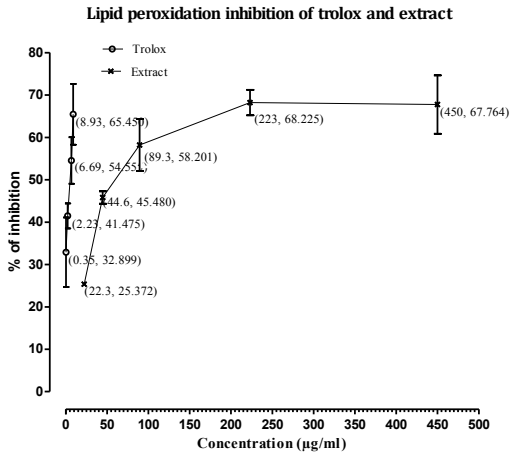

Fig. 3. The percentage of inhibition of the non-enzymatic lipid peroxidation by trolox (n=3) and extract (n=2). Each value is expressed as mean ± SEM.

IV. Conclusion

The *n*-hexane extract of *J. curcas* L. leaf was evaluated for its antioxidant activity by three methods based on hydrogen donating ability, electron transfer capacity and hydroxyl radical scavenging activity. In lipid peroxidation assay, the extract expressed a stronger antioxidant activity, compared with the other two methods. The non-polar compound in this extract especially

phytosterols such as β-sitosterol was proposed to be responsible for this potent inhibition on lipid peroxidation. However, the β-sitosterol content in this extract and the antioxidant activity of *n*-hexane extract of *J. curcas* L. leaf in parallel to its inhibition of lipid peroxidation should be established prior to conclude that β-sitosterol plays an important role in lipid peroxidation inhibition induced by *n*-hexane extract of *J. curcas* L. leaf.

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